Peptide Bond Formation on an Oligonucleotide Template

J. Bitterman, P. Marguet, T. Halo, J. Stambough, A. Cramer and D. Usher
Department of Chemistry and Chemical Biology
Cornell University, Ithaca, NY 14853-1301

Much of the recent work that has been done on the selection of RNA catalysts has made use of contemporary enzymes to copy and amplify interesting sequences. These enzymes are very good at making 3'-5' internucleotide bonds, but there is no obvious reason why the structures of prebiotic RNA catalysts should have been so circumscribed. Thus our present work on oligonucleotide-directedpeptide bond formation uses a template molecule in which two oligonucleotides are joined head-to-head by a 5',5'-pyrophosphatebond. This type of structural feature is found today in the coenzymeNAD and (as a triphosphate) in the eukaryotic mRNA cap, and has turned up, apparently unexpectedly, in some ribozyme experiments(1).

Examination of the crystal structure(2) of the transition-stateanalog CCdA–p–Pur, bound to the 50S ribosomal subunit of *Haloarcula marismortui*, suggested that two oligonucleotides, joined by a 5',5'-pyrophosphate bond, may be able to act as a template for the formation of peptide from two 2'(3')-aminoacyl oligonucleotides that are bound to the template with their 3'-termini juxtaposed.

Our first experiments made use of the template 3'-UGGU-5'-pp-5'-UGGU-3'and the complementary ACCA, which was aminoacylated at the 3'-terminus.However, complex formation between these two molecules was never satisfactorily demonstrated, so we moved to a longer sequence:3'-GACUGU-5'-pp-5'-UGUCAG-3' and the complement 5'-CUGACA-3'.The melting temperature of a 1:2 ratio of these two components(in 0.5 M NaCl at pH 7.25) was 40°C, a convenient temperature at which to investigate possible peptide bond formation. Synthesis of the template and aminoacylation of its complement was greatly simplified by the judicious use of 2'–OMe RNA.

Our original hope was to use reverse-phase HPLC with UV detection at 205 nm to look for peptide bond formation, but interference by nucleotidic material proved too severe. We are now investigating the use of Sanger's reagent, with analysis by HPLC and detection at 400 nm, to quantify any dipeptide that is formed. An alternative method, if Sanger's reagent is unsuccessful, will be to use a C-14 labelled amino acid.

(1) K.B. Chapman and J.W. Szostak, *Chem. Biol.* **2**, 325-333 (1995); F.Q. Huang and M. Yarus, *PNAS* **94**, 8965-8969 (1997).

(2) N. Ban et al., *Science* **289**, 905-920 (2000).